INHIBITION OF FERTILIZIN AGGLUTINATION OF SPERM BY THE DERMAL SECRETION FROM ARBACIA [1]

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Upon appropriate stimulation, *Arbacia* release a yellowish fluid from the integument. This fluid strongly inhibits fertilization (Oshima, 1921; Pequegnat, 1948) and is probably the material responsible for the inhibitory action ascribed to *Arbacia* blood by Lillie (1914). Current interest arose from the observation that this "dermal secretion" not only inhibits fertilization but inhibits fertilizin agglutination of sperm as well. Fertilizin is the specific sperm isoagglutinin obtained from eggs of the species. It is initially present as a jelly surrounding the sea urchin egg, but on standing in sea water this jelly dissolves charging the water with the agglutinin. The dual effect of the dermal secretion suggests a causal relation between inhibition of fertilizin agglutination of sperm and of fertilization. Demonstration of such a relation would support the view that fertilizin is essential for fertilization. The experiments described here and reported briefly elsewhere (Metz, 1958) mainly concern the action of the dermal secretion on sperm and the fertilizin agglutination of sperm. The results are consistent with the view that inhibition of fertilization by the dermal secretion is related to an inhibitory action on fertilizin.

Materials and Methods

Arbacia punctulata was used in all experiments except the specificity tests outlined in the text. Most of this material was collected in the vicinity of the Florida State University Marine Laboratory, Alligator Point, Florida, although a few collections were made from a very large population at Panama City, Florida. A few experiments were performed at the Marine Biological Laboratory, Woods Hole, Mass., using the local material.

Gametes were obtained by electrical stimulation of the animals. Gametes obtained by the KCl injection method, especially during the winter months, proved unsatisfactory in both fertilization and sperm agglutination tests (Metz, 1957a). In fact, investigation of the cause of this failure revealed that KCl-treatment frequently stimulated release of the dermal secretion (see also Harvey, 1956, page 57) as well as the gametes.

Sperm oxygen consumption was measured using standard Warburg apparatus. Single side arm vessels of 15–20 ml. capacity containing 3 ml. of fluid were run in duplicate at 20° C. The vessels were shaken at a rate of 120 cycles per minute with an amplitude of 3.5 cm.

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Solutions of the dermal secretion were prepared in a manner similar to that described by Pequegnat (1948), and consisted of the following steps: The *Arbacia* were immersed in tap water for 1–3 minutes. They were subsequently rinsed in distilled water and finally in filtered sea water. The animals were then placed in a dry funnel and the yellow fluid which drained off was collected. The pH of such fluid is about 7.5. In experiments where this might be critical the pH was raised to that of sea water (pH 8.0–8.2). To test for sperm agglutination one to two drops of the test solution were mixed with an equal volume of a sperm suspension prepared by diluting semen to 2% with sea water. The mixtures were examined both macro- and microscopically.

Results

A) *Action on sperm*

Pequegnat (1948) noted that the dermal secretion of *Arbacia* stimulated sperm to increased activity. This effect was observed repeatedly in the current investigation. Even preparations of dermal secretion at the initial pH (7.5), and therefore at a pH disadvantage as compared to sea water controls, had the stimulating action. As Pequegnat was aware, this effect is not a transient one. The sperm remain strongly active for a prolonged period of time.

The enhanced motility of the sperm in the presence of the dermal secretion is associated with an increase in oxygen consumption as seen in Figure 1. Again the effect is not a short term one for the inhibitor-treated sperms were still respiring at approximately twice the rate of the controls even at the end of one hour. The increased oxygen uptake is clearly the result of enhanced sperm respiration, not to the oxidation of the pigment in the dermal secretion (see later section). This follows from the fact that the increase in oxygen uptake does not appear until the dermal secretion is tipped into the sperm suspension. Furthermore, in independent experiments dermal secretion collected over nitrogen to prevent premature oxidation failed to consume appreciable amounts of oxygen in Warburg vessels.

Nothing definite is as yet known regarding the chemical nature or properties of the sperm-stimulating agent, except that it is gradually destroyed by heat (Fig. 1).

B) *Action on fertilizin agglutination of sperm*

An inhibitory effect of the dermal secretion on fertilizin agglutination of sperm was first noted by Pequegnat (1948). The present investigation was designed to obtain information concerning the mechanism of action of the material in this effect. Attention was directed especially to determine if the inhibitory agent acted upon the sperm, the fertilizin or both constituents of the agglutinating system.

Possible inhibitory action on the sperm. One possible means whereby the dermal secretion could inhibit agglutination would be by action on the sperm. For example, the agent could destroy the antifertilizin receptor sites on the sperm surface. Such destruction might be expected to be irreversible in which case washing dermal secretion-treated sperm should not restore agglutinability. Such experiments show, however, that treated sperm recover agglutinability upon wash-

ing in sea water. A typical experiment from a series of four is given in Table I. From this experiment it is clear that the inhibiting agent in the dermal secretion does not render the sperm irreversibly refractory to the agglutinating action of fertilizin.

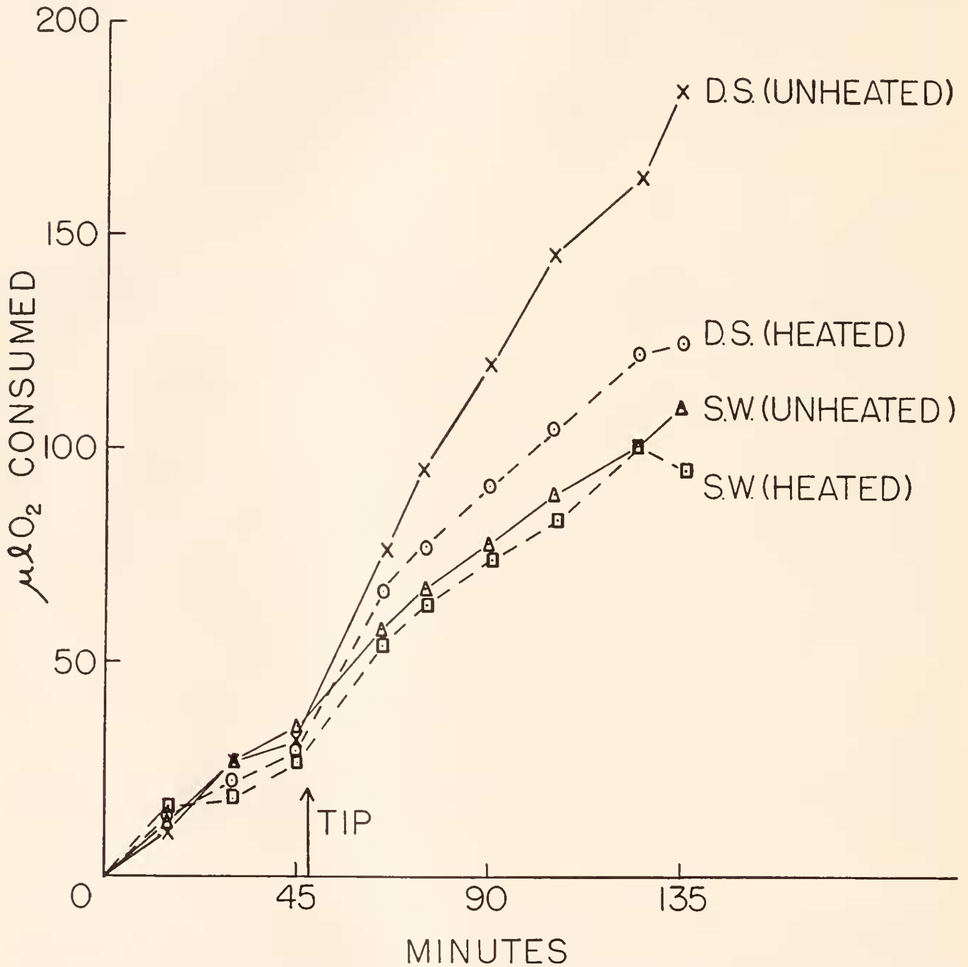

FIGURE 1. Effect of dermal secretion (D.S.) on oxygen uptake of *Arbacia* sperm. Heated aliquots (3 ml.) were immersed in a boiling water bath for 5 minutes. The pH of both heated and unheated dermal secretion solutions was adjusted to 7.9. Vessels contained 1.8 ml. of *Arbacia* semen diluted to 10% with sea water. Center wells contained 0.2 ml. 20% KOH. The 1-ml. samples of dermal secretion (or sea water) in side arms were tipped into vessels at 48 minutes.

Likewise, the inhibitor is not used up or destroyed by sperm in the inhibiting reaction as seen in Table II (one of five experiments). Accordingly, it appears likely that the dermal secretion acts upon the fertilizin, not the sperm, to inhibit agglutination. Experiments supporting this view are described below.

TABLE I

Fertilizin agglutination of Arbacia sperm after washing from dermal secretion

	1	2	3	4
	Sperm + D.S. (Sperm re-suspended in S.W.)	Sperm + D.S. (Sperm re-suspended in original supernatant D.S.)	Sperm + S.W. (Sperm re-suspended in S.W.)	Sperm + S.W. (Sperm re-suspended in original supernatant S.W.)
Agglutination of sperm on addition of fertilizin after one centrifugation	+	−	++++	++++
Agglutination of sperm on addition of fertilizin after second centrifugation	+++	−	++++	++++

Semen concentration in the four 0.75-ml. samples was 8%. After standing 30 minutes the samples were centrifuged twice at 4° C. Sperm in 1 and 3 was re-suspended in sea water; the sperm in 2 and 4 in the original supernatant after each centrifugation. Aliquots of sperm from all re-suspended samples were tested for agglutination with fertilizin.

Inhibitory action on fertilizin. Tests for action of the inhibiting solution on fertilizin are complicated by the fact that fertilizin itself can inhibit sperm agglutination when in the univalent form (Metz, 1942). Indeed, one possible mode of action of the inhibitor would be that it converts fertilizin from the multivalent, agglutinating form to the univalent, non-agglutinating condition. Another pos-

TABLE II

Agglutination inhibiting action of sperm absorbed dermal secretion

	Absorbing mixtures			
	1	2	3	4
0.5 ml.	Sperm (33%)	S.W.	Sperm (33%)	S.W.
	+	+	+	+
0.5 ml.	D.S.	D.S.	S.W.	S.W.
	Agglutination inhibiting assay of supernatants from above mixtures			
Dilution of absorption supernatant	1	2	3	4
1	−	−	++++	++++
$\frac{1}{2}$	−	−		
$\frac{1}{4}$	±	−		
$\frac{1}{8}$	+++	++		
$\frac{1}{16}$	+++	+++		

After standing for 30 minutes the absorbing mixtures were centrifuged, supernatants were removed and diluted serially in S.W. Constant amounts of fertilizin and test sperm suspension were then added to each dilution. The slight difference between No. 1 and No. 2 is not considered significant.

sibility is that the inhibitor destroys the reactive sites of the fertilizin which combine with the sperm surface. This could occur with or without conversion to the univalent form.

Ideally, to test for direct action on fertilizin, the inhibiting agent and fertilizin should be mixed and subsequently separated before testing the fertilizin for activity. Unfortunately, no simple means has yet been devised for separating the two materials. However, results obtained using two other procedures leave no doubt that the agglutination-inhibiting agent does destroy not only the sperm-agglutinating action of fertilizin, but also the ability of the fertilizin to combine with the anti-fertilizin of the sperm.

In the first procedure advantage was taken of the fact that the agglutination-inhibiting action of the dermal secretion is rapidly destroyed by heating to 100° C.

TABLE III

Effect of heating on fertilizin-dermal secretion mixtures

	Heated mixtures				Unheated mixtures			
	1	2	3	4	5	6	7	8
0.5 ml. + 0.5 ml.	D.S. + fertilizin	D.S. + S.W.	S.W. + fertilizin	S.W. + S.W.	D.S. + fertilizin	D.S. + S.W.	S.W. + fertilizin	S.W. + S.W.
A. Agglutination on addition of sperm to mixtures	–	–	++++	–	–	–	++++	–
B. Inhibition tests (agglutination on addition of control fertilizin to samples tested in *A* above)	+++	+++	–	+++	–	–	–	+++

The heated mixtures were held at 100° C. for four minutes. In *A* the mixtures were tested for agglutinating action on sperm. The heating failed to restore agglutinating action to the D.S.-fertilizin mixture (*A1*). In *B* the mixtures were tested for agglutination inhibiting action by adding control fertilizin to the mixtures in *A* following spontaneous reversal of the initial agglutination in *A3* and *A7*. The heated mixture fails to inhibit agglutination (*B1* and *B5*). Both the inhibitor in the dermal secretion and the fertilizin have been destroyed in the heated mixture.

whereas fertilizin is relatively stable to such heating. As seen in Table III (A) when a non-agglutinating, fertilizin-inhibitor mixture was heated sufficiently to destroy the inhibitor but not the fertilizin, the mixture still failed to agglutinate sperm. Restoration of agglutinating action would be expected if the inhibitor in the mixture acted exclusively on the sperm. Accordingly, the experiment is explained by an inactivation of the fertilizin by the inhibitor and a subsequent inactivation of any remaining inhibitor by the heating. Four other experiments yielded similar results.

The question now arises whether the agent inactivates the sperm combining sites of fertilizin or converts the fertilizin to the univalent form without combining site destruction. Evidence concerning this was obtained by testing for sperm

agglutination inhibiting action of the heated fertilizin-inhibitor mixture. As seen in Table III (B) control fertilizin agglutinates sperm that was pretreated with the heated fertilizin-inhibitor mixture. This agglutination means that the heated mixture contains insufficient fertilizin combining sites to block the sperm surface. However, controls in the experiment show that fertilizin combining sites sufficient to block the sperm surface were initially present in the mixtures. Therefore, it must be concluded that combining site destruction occurred in the fertilizin-inhibitor mixture. Inactivation of agglutinating action by dermal secretion does not result simply from conversion of the fertilizin to the univalent form.

In a second series of experiments fertilizin was tested for its ability to combine with the sperm surface in the presence of unheated dermal secretion. As seen in Table IV sperm was mixed with a non-agglutinating inhibitor-fertilizin mixture in proportions that assured sufficient fertilizin to saturate the sperm surface. The sperm in the mixture was subsequently centrifuged free of the mixture and tested

Table IV

Fertilizin agglutination of Arbacia sperm washed from fertilizin-dermal secretion mixtures

	1	2	3	4
Sperm washed from mixture of.....	D.S. + fertilizin	D.S. + S.W.	S.W. + fertilizin	S.W. + S.W.
Agglutination on addition of fertilizin to the washed sperm	+++	++++	−	++++

The four mixtures were prepared in the following proportions: 0.5 ml. dermal secretion, 0.25 ml. fertilizin, 0.25 ml. approximately 47% semen. They were centrifuged at 4° C., and the sperm were re-suspended in 0.5 ml. sea water. These sperm suspensions were then tested for agglutinability with control fertilizin. The sperm suspensions were not agglutinated in sea water alone. Test 3 shows that the original mixtures 1 and 3 contained sufficient fertilizin to block the sperm surface. In the presence of dermal secretion, this amount of fertilizin failed to block the sperm surface (test 1). Controls not listed show that the mixtures contained an excess of dermal secretion.

for agglutinability by control fertilizin. As seen in the table the sperm washed from the fertilizin-inhibitor mixture agglutinated upon addition of control fertilizin, but sperm washed from fertilizin alone failed to agglutinate. In the latter case, the fertilizin evidently saturated the sperm surface (as univalent fertilizin) to prevent agglutination by the control fertilizin. Failure of fertilizin to block the sperm surface in the fertilizin-inhibitor mixture is most readily explained by an inactivation of combining sites of fertilizin by the inhibitor prior to addition of sperm.

C) *Action on antifertilizin agglutination of eggs*

In view of its action on solutions of fertilizin the dermal secretion might be expected to affect fertilizin in the gel form. The natural jelly surrounding the eggs of sea urchins is largely, if not entirely, fertilizin (Tyler, 1949). Thus the dermal secretion might be expected to precipitate, dissolve or otherwise alter this

jelly layer in some visible way. In any event it should affect the egg agglutination and jelly precipitation reaction that results when antifertilizin from sperm is mixed with eggs.

According to Pequegnat (1948) (p. 79) the dermal secretion "appeared to remove part of the egg's jelly layer, in proportion to concentration or to the duration of exposure." Similar action was sometimes observed in the present investigation. However, the effect was not consistently found. It seems likely that this jelly-dissolving action is related to the pH of the inhibitor solutions. Aside from this possible dissolving action the dermal secretion has no visible effect on the egg jelly. It certainly does not precipitate the egg jelly in the form of a membrane as might be expected in view of its ability to inactivate the combining sites of fertilizin.

In spite of its failure to have a direct precipitatory action on the egg jelly, the dermal secretion was found to inhibit the jelly precipitation and egg agglutination

TABLE V

Effect of heated and unheated dermal secretion on A, fertilizin agglutination of sperm and B, antifertilizin precipitation of egg jellies

	A				B			
	D.S. + fertilizin + sperm	D.S. + S.W. + sperm	S.W. + fertilizin + sperm	S.W. + S.W. + sperm	D.S. + anti-fertilizin + eggs	D.S. + S.W. + eggs	S.W. + anti-fertilizin + eggs	S.W. + S.W. + eggs
Unheated D.S.	−	−	++++	−	−	−	++++	−
Heated D.S.	++++	−	++++	−	−	−	++++	−

The heated dermal secretion was treated for 5 minutes at 100° C. Two other experiments gave similar results. In one of these the dermal secretion was heated for 25 minutes.

that results when sperm antifertilizin is mixed with eggs. However, the inhibiting action on egg jelly precipitation was found to be reversible. After washing the eggs from the dermal secretion or dermal secretion-antifertilizin mixture into sea water, and subsequently adding antifertilizin they formed satisfactory jelly precipitation membranes. Clearly, then, the dermal secretion inhibits antifertilizin agglutination of eggs. However, the mechanism of this action is apparently more complicated than would have been predicted from the mode of action of the dermal secretion on sperm agglutination. Results from the latter study (above) indicate that the dermal secretion inactivates the combining sites of fertilizin. The inhibition of egg agglutination by antifertilizin, however, appears to result primarily from action of the material on the egg agglutinating agent, antifertilizin, in the sperm extracts. This conclusion is suggested by 1) the ready reversibility of the inhibition upon washing eggs, 2) the fact that a precipitate forms upon mixing antifertilizin and the dermal secretion and finally, 3) the fact that the egg agglutination

inhibitor is heat-stable, whereas the fertilizin agglutination inhibitor is heat-labile. These last relations are demonstrated in the experiment summarized in Table V. From these experiments it would appear that there are two inhibitors in the dermal secretion. One of these inhibits the sperm agglutinating action of fertilizin by inactivating the combining sites of the agglutinin. The second agent inhibits the egg jelly precipitating and agglutinating action of sperm antifertilizin by inactivating the antifertilizin.

Although these experiments adequately explain failure of antifertilizin to agglutinate eggs in the presence of dermal secretion, they still do not directly answer the question whether the heat-labile agent can combine with the reactive sites of fertilizin when the latter is in the gel form. To test for such combination, eggs were treated with an excess of dermal secretion for periods up to 55 minutes. The eggs were subsequently washed in sea water and extracted for fertilizin in normal or acid sea water. In each of three such experiments the dermal secretion-treated eggs yielded sperm agglutinating fertilizin solutions. Indeed the sperm agglutinin titers of fertilizin solutions prepared by acid extraction of dermal secretion-treated and control eggs were not significantly different. Evidently, then, the reactive sites of fertilizin in the gel form are not accessible to the heat-labile inhibitor of the dermal secretion.

D) *Some physical and chemical properties of the dermal secretion*

No systematic study of the physical and chemical properties of the dermal secretion has yet been made. Nevertheless, some information has been obtained concerning the secretion and the sperm agglutination inhibitor contained in it. This information is recorded here.

Color changes. The freshly prepared dermal secretion is light yellow-green in color. Upon standing in air the solution gradually darkens to a deep brown or black color. This color change is evidently a rather direct oxidation by atmospheric oxygen, for the color change fails to occur in an atmosphere of nitrogen. Furthermore, it appears unlikely that the oxidation is mediated by enzymes because both heated and unheated preparations undergo the color changes. Finally, the oxidation is not reversed by reducing agents such as hydrosulfite. The colored material is a component of a large molecule since it precipitates with $(NH_4)_2SO_4$ and fails to diffuse through cellophane. The sperm agglutination inhibitor is associated with the pigmented material to the extent that the inhibitor also precipitates with $(NH_4)_2SO_4$ and fails to diffuse.

Antigenic composition. No serious serological study has been made of the dermal secretion. Nevertheless, the dermal secretion lowered the sperm agglutinating titer of anti-*Arbacia* sperm serum. In agar diffusion precipitin tests (Ochterlony tests) the dermal secretion produced three precipitin bands both with antisera prepared against *Arbacia* sperm and with antisera against jellyless *Arbacia* eggs. One precipitin band formed when the dermal secretion was diffused against antiserum prepared against *Arbacia* fertilizin. No precipitates formed with control (pre-injection) serum. The antisera used in these experiments were prepared with considerable care. The sperm and jellyless eggs used for injection were washed to remove contaminating material. Likewise, the fertilizin used as immunizing antigen was obtained by careful acid extraction of washed eggs (see Tyler, 1949).

In view of this, it appears likely that the immunizing antigens were largely free of contaminating material including dermal secretion. Evidently, then, the dermal secretion contains antigenic groups in common with sperm, eggs and fertilizin. The antigenic relationships here have not yet been investigated, but it is evident that the dermal secretion contains at least three separate and distinct materials.

Discussion

The observations of Oshima (1921) and Pequegnat (1948) combined with the present investigation show that the dermal secretion of *Arbacia* has a variety of effects on sea urchin gametes and their interaction. Action on sperm includes enhancement of motility and respiration. These are not pH effects but appear to depend upon an agent or agents in the dermal secretion. The two effects may be due to separate agents although it seems more likely that increase in motility and respiration are related and result from action of a single agent. Little information concerning the mechanism of action of the agent has been obtained. However, it is unlikely that the motility and respiration enhancing effects are due to a metal-binding action of the dermal secretion. Metal-binding agents do increase the motility of sea urchin sperm (Tyler and Atkinson, 1950), but they differ from the dermal secretion in that they fail to increase the rate of oxygen uptake (Tyler and Rothschild, 1951; Tyler, 1953). Metal-binding agents and the dermal secretion of *Arbacia* also differ in their action on *Asterias* sperm. The former agents have a spectacular stimulatory action on *Asterias* sperm motility (Metz and Birky, 1955) whereas the dermal secretion of *Arbacia* was observed to have no effect on *Asterias* sperm motility. Evidently, the dermal secretion does not contain appreciable amounts of substances which bind metals. Finally, contamination with the dermal secretion is a hazard to be avoided in studies of stimulating action of egg water and other extracts on sperm motility and respiration.

The dermal secretion also inhibits fertilizin agglutination of sperm, antifertilizin precipitation of egg jelly and fertilization. From the sperm centrifugation experiments it is evident that the dermal secretion does not inhibit agglutination by combining with and blocking the sperm surface in irreversible fashion. In this respect the agent differs from fertilizin. As is well known, sea urchin sperm washed from an excess of fertilizin after reversal of agglutination fails to reagglutinate upon a second addition of fertilizin. Indeed, sperm remove the fertilizin from solution (*e.g.,* Monroy *et al.,* 1954). Conceivably, the agent could inhibit agglutination enzymatically by digesting the antifertilizin from the sperm surface with sufficient rapidity to prevent agglutination. If the antifertilizin were arranged in layers at the sperm surface, sufficient of this material might remain after addition and subsequent removal of the inhibitor to insure agglutination by fertilizin. However, even granting this unlikely possibility it is clear from the experiments with dermal secretion-fertilizin mixtures that the dermal secretion destroys the agglutinating action of fertilizin. In the first series of these experiments non-agglutinating mixtures were heated sufficiently to destroy the inhibiting agent but not the fertilizin. Such heated mixtures failed to agglutinate sperm and to inhibit agglutination. In the second series sperm were washed from unheated inhibitor-fertilizin mixtures containing sufficient fertilizin to block the sperm sur-

face. These washed sperm agglutinated on addition of fertilizin. These experiments show that the dermal secretion of *Arbacia* can inhibit agglutination by inactivation of the agglutinin fertilizin. This inactivation is not simply a conversion of the fertilizin molecule to a form similar to the univalent fertilizin produced by some other agent (see Metz, 1957b, for review). The inhibiting agent in the dermal secretion inactivates or blocks the combining sites of the fertilizin that react with the sperm surface in agglutination.

In view of this action on fertilizin it is surprising that the dermal secretion has no visible effect on the sea urchin egg jelly since this jelly consists of undissolved fertilizin. It is even more surprising that eggs washed from dermal secretion will agglutinate with antifertilizin, since the agent in the dermal secretion inactivates the combining sites of fertilizin. These unexpected observations are explained by experiments showing that the combining sites of fertilizin are not inactivated by dermal secretion when the fertilizin is in the gel form. Possibly the inhibiting agent fails to diffuse through the egg jelly. However, it seems more likely that the specific combining sites are inaccessible to the dermal secretion because they are blocked by cross linkages in the gel structure. This concept has been offered as a possible explanation of passage of sperm through the egg jelly without saturation by fertilizin (Tyler, 1941). It is also consistent with the observation that fertilizin in solution and in the gel form differs in staining properties with metachromatic dyes (Monroy *et al.*, 1954). Even with this explanation one difficulty remains. If the combining sites of fertilizin in the gel form are not available for reaction with dermal secretion, then they might reasonably be expected to be unavailable to antifertilizin as well. This difficulty is resolved by assuming that egg jelly precipitation results from combination of antifertilizin with a part of the fertilizin other than the specific combining sites involved in sperm agglutination.

Finally, consideration of the effect of dermal secretion on the egg jelly precipitating action of antifertilizin leads to other interesting implications. In the presence of dermal secretion antifertilizin fails to precipitate egg jellies. This inhibition results from a second, heat-stable agent in the dermal secretion which acts upon antifertilizin. This complicates the problem further because the first experiments performed in this study show that the dermal secretion does not irreversibly inactivate the combining sites of the sperm surface antifertilizin. Therefore, the heat-stable inhibitor must inactivate egg agglutinating antifertilizin preparations by combination with some other part of the molecule. A final possibility that is not excluded is that the "antifertilizin" extracted from sperm is not related to the sperm surface material that combines with fertilizin in the sperm agglutination reaction. The "antifertilizin" may be a fortuitous product of the extraction procedure which, like some other proteins, precipitates sea urchin egg jelly nonspecifically.

One other question requires consideration; namely, the relation of the inhibitors described above to inhibition of fertilization. Like the sperm agglutination inhibitor the fertilization inhibitor is heat-labile. This is consistent with the view that inhibition of agglutination and of fertilization result from action of the same agent. Further investigation of this relationship may reveal that the fertilization inhibiting action of dermal secretion results from inactivation of fertilizin.

Other agents are also known to inhibit fertilization in the sea urchin. Notable among these are the preparations from the brown alga, *Fucus,* studied by Runnström and co-workers. Two inhibitory agents are recognized. One is a phenolic substance and has not been studied in great detail (Wicklund, 1954). The other, named "fertilization inhibitor (Fucus)," is heat-stable, increases motility of sperm (Wicklund, 1954) but does not inhibit fertilizin agglutination of sperm (Runnström and Hagstrom, 1955). Clearly this agent has different properties than the dermal secretion and the two preparations probably act in different fashion to inhibit fertilization. Indeed, they may very well block different steps in the initial stages of fertilization. Accordingly, a detailed analysis of the site and mechanism of action of these and other inhibitors might provide interesting information concerning the sequence of events in the initial stages of fertilization.

Summary

1. As demonstrated previously by Oshima (1921) and Pequegnat (1948) *Arbacia* release a yellowish secretion upon appropriate stimulation.

2. This dermal secretion increases the motility and oxygen consumption of sperm. The effect is not short lived and does not appear to depend upon a metal binding action.

3. The dermal secretion inhibits fertilizin agglutination of sperm. This action results from an inactivation of the specific combining sites of fertilizin. The dermal secretion does not act upon the sperm surface to inhibit agglutination. The agent in dermal secretion that inactivates fertilizin is destroyed by heating to 100° C. and fails to diffuse through cellophane.

4. The dermal secretion has no visible action on the *Arbacia* egg or egg jelly.

5. The dermal secretion inhibits antifertilizin precipitation of intact egg jellies. This action depends upon a heat-stable agent which precipitates antifertilizin.

6. Some properties of the dermal secretion are described. The material contains at least three distinct antigens.

7. The results are in agreement with the view that fertilizin is essential for fertilization.

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